Virus Genes
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Visualization of Marek's disease virus in vitro using enhanced green fluorescent protein fused with US10

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Abstract Marek's disease virus (MDV) is an highly cell-associated avian alphaherpesvirus. Although viral replication is supported in chicken embryo fibroblasts (CEF) or duck embryo fibroblasts, identification of MDV-infected cells is quite cumbersome especially during the early stages of virus replication when plaques can be difficult to recognize. To visualize MDV replication in infected cells and characterize MDV US10 in vitro, rMd5-US10-EGFP, a recombinant MDV, was generated that expresses enhanced green fluorescent protein (EGFP) as a tagged protein fused with US10 at the C-terminal end. The expression of US10-EGFP was detected in infected CEF using fluorescent microscopy and the expression intensity was quantified using flow cytometry analysis. In addition, confocal microscopic analysis provided information on subcellular localization of US10-EGFP in virus-infected cells. In conclusion, rMd5-US10-EGFP virus can be used to help monitor virus activity in vitro.

Keywords Marek's disease virus · US10 · Enhanced green fluorescent protein · Bacterial artificial chromosome

Weifeng Mao and Taejoong Kim contributed equally to this study.

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Marek's disease (MD) is a lymphoproliferative disease in chickens with susceptible birds developing lymphomas in visceral organs and nerve enlargement as early as 6 weeks postinfection [1]. The causative agent, Marek's disease virus (MDV), belongs to the *Alphaherpesvirinae* subfamily but unlike the other members, MDV does not make extracellular virions in cell culture or viremic serum from infected chickens [2]. Because MDV is highly cell-associated, identifying a virus-infected cell, especially during the early lytic replication stage, is difficult and typically, staining of viral antigens is required for visualizing MDV plaques in cell culture.

MDV US10 encodes a 213 amino acid protein that is not essential for growth in chicken embryo fibroblast (CEF) culture and, based on 33 % amino acid homology to herpes simplex virus US10, is proposed to be a capsid/tegument-associated phosphoprotein [3–5]. With the recent availability of infectious MDV bacterial artificial chromosome (BAC) clones and significant improvements in BAC clone manipulations [6–12], MDV ORFs such as US10 can be modified and their role in MDV pathogenesis can be directly examined via mutagenesis. In this study, the C-terminal end of US10 was fused with EGFP using the rMd5-B40 BAC clone [9] and BAC modification techniques (Supp. Fig. 1a.). Comparison of the overall digestion patterns with the parental rMd5-B40 did not indicate any unexpected modifications elsewhere in the MDV BAC (Supp. Fig. 1b), and the virus expressed the US10-EGFP fusion protein with the expected size as determined by Western blot analysis (Supp. Fig. 1c).

The resulting recombinant MDV was used to infect CEF. Once visible viral plaques were observed, EGFP expression could be readily monitored through an inverted fluorescent microscope. The EGFP expression pattern corresponded with the viral plaques as shown in Fig. 1. In

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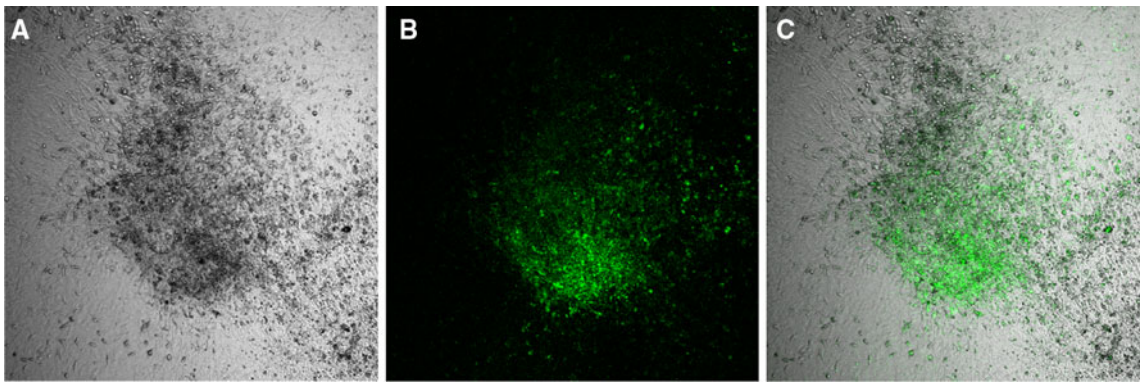

Fig. 1 Fluorescence analysis of CEF infected with the rMd5-US10-EGFP virus. The recombinant MDV was generated by inserting a 946 amplicon-containing EGFP inframe to the C-terminal end of US10 of the rMD5-B40 BAC clone. The resulting clone was transfected into CEF and monitored through an inverted fluorescent microscope at 3 dpi. **a** Infected CEF was visualized through differential interference contrast (DIC) imaging. **b** EGFP-US10 was visualized via EGFP fluorescence (*green*). **c** The merged image of a and b

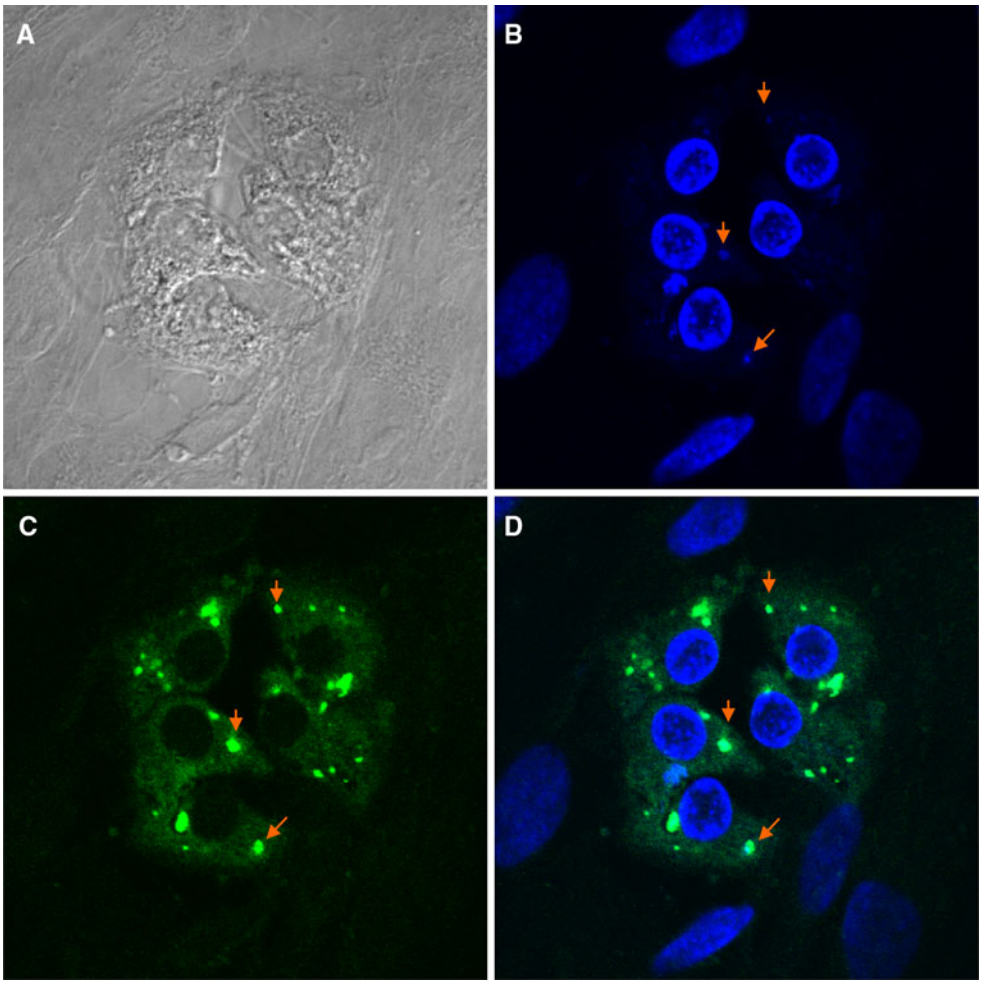

Fig. 2 Subcellular localization of viral US10-EGFP in rMd5-US10-EGFP virus-infected cells. The recombinant MDV with an US10-EGFP fusion protein was used to infect CEF, then visualized using confocal microscopy at 3 dpi. **a** The infected CEF was visualized through differential interference contrast (DIC) imaging. **b** rMd5-US10-EGFP virus-infected CEF culture with Hoechst 33342 staining; arrows show DNA staining in the cytoplasm. **c** US10-EGFP was visualized through EGFP fluorescence; arrows show US10-EGFP in the cytoplasm. **d** Merged image of b and c; arrows show colocalization of US10-EGFP and cytoplasmic DNA

addition, EGFP expression was detectable at 3 dpi by flow cytometry analysis and increased in signal strength to 5 dpi (Supp. Fig. 2a), which suggested that the US10-EGFP MDV could be potentially used to detect the early stage of infection. This suggestion is supported by the observation that we could detect staining in adjacent cells that did not show obvious signs of cytopathic effects; MDV is highly cell-associated and typically not synchronized, therefore, viruses in culture are at various stages of infection. The parental and mutant viruses did not show obvious growth differences in vitro (Supp. Fig. 2b).

The characterization of MDV US10 in virions is not known, although it has been predicted as a capsid/tegument phosphoprotein based on HSV-1 US10. HSV-1 US10 is localized in the nucleus though low amounts of the protein are also observed in the cytoplasm [13, 14]. Using our recombinant MDV with an US10-EGFP protein fusion, we determined that MDV US10 has a subcellular localization in the cytoplasm in all our observations (Fig. 2). As shown, US10-EGFP accumulates in the cytoplasm and colocalizes with cytoplasmic DNA which is consistent with an earlier report [15]. The localization of US10 to the cytoplasm is unlikely due to the addition of EGFP as another recombinant MDV that expresses EGFP only displays fluorescence evenly distributed in both the cytoplasm and nucleus of infected cells (Supp. Fig. 3). The difference in localization between MDV and HSV-1 is unclear though the genes are not collinear and, as mentioned, exhibit limited (33 %) amino acid similarity [16].

In conclusion, the fusion of EGFP with MDV ORFs through BAC modification is a method for detecting viral infection and for visualizing the subcellular localization of viral proteins such as MDV US10 localized in the cytoplasm. As MDV US10 was reported to interact with SCA2, the product of an MD resistant gene, we hope that the US10-EGFP MDV may be useful to understand the chicken SCA2-MDV US10 protein–protein interaction using cultured chicken cells as an initial model [17].

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References

1. S.J. Baigent, F. Davison, *Marek's Disease: An Evolving Problem* (Elsevier, London, 2004), pp. 62–77
2. K. Osterrieder, J.-F. Vautherot, *Marek's Disease: An Evolving Problem* (Elsevier, London, 2004), pp. 17–31
3. B. Lupiani, L.F. Lee, S.M. Reddy, Curr. Top. Microbiol. Immunol. **255**, 159–190 (2001)
4. M.S. Parcells, A.S. Anderson, J.L. Cantello, R.W. Morgan, J. Virol. **68**, 8239–8253 (1994)
5. M.S. Parcells, A.S. Anderson, R.W. Morgan, J. Virol. **69**, 7888–7898 (1995)
6. D. Schumacher, B.K. Tischer, W. Fuchs, N. Osterrieder, J. Virol. **74**, 11088–11098 (2000)
7. L. Petherbridge, A.C. Brown, S.J. Baigent, K. Howes, M.A. Sacco, N. Osterrieder, V.K. Nair, J. Virol. **78**, 13376–13380 (2004)
8. S.J. Baigent, L.P. Smith, R.J. Currie, V.K. Nair, J. Gen. Virol. **87**, 769–776 (2006)
9. M. Niikura, J.B. Dodgson, H.H. Cheng, Arch. Virol. **151**, 537–549 (2006)
10. E.C. Lee, D. Yu, J. Matinez de Velasco, L. Tessarollo, D.A. Swing, D.L. Court, N.A. Jenkins, N.G. Copeland, Genomics **73**, 56–65 (2001)
11. S. Warming, N. Costantino, D.L. Court, N.A. Jenkins, N.G. Copeland, Nucleic Acids Res. **33**, e36 (2005)
12. B.K. Tischer, J. von Einem, B. Kaufer, N. Osterrieder, Biotechniques **40**, 191–197 (2006)
13. J. Salsman, N. Zimmerman, T. Chen, M. Domagala, L. Frappier, PLoS Pathog. **4**, e1000100 (2008)
14. J. Xing, S. Wang, Y. Li, H. Guo, L. Zhao, W. Pan, F. Lin, H. Zhu, L. Wang, M. Li, L. Wang, C. Zheng, Med. Microbiol. Immunol. **200**, 61–68 (2011)
15. M.S. Parcells, R.L. Dienglewicz, A.S. Anderson, R.W. Morgan, J. Virol. **73**, 1362–1373 (1999)
16. B. Lupiani, L.F. Lee, S.M. Reddy, *Marek's Disease* (Springer, New York, 2001), pp. 159–190
17. H.C. Liu, M. Niikura, J. Fulton, H.H. Cheng, Cytogen. Genome Res. **102**, 304–308 (2003)